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The Amino Acid Composition of Sweat in Cystic Fibrosis of the Pancreas as Determined by the Ninhydrin-CO_2 Method.

by

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INTRODUCTION

Although investigations of the contents of sweat may be traced back a great many years, the last fifteen years have seen a rapidly increasing interest in the subject. Formerly, research in this field was concerned to a great extent with the adaptation of the body to climate. Recently, though, two findings have been reported which indicate that the sweat glands respond to varied changes in the physiologic state, and may serve as an excellent site of study to yield further information about metabolic derangements. One of the reports which has stimulated interest in the sweat gland was made by Conn and his group in 1946,[1] when they indicated that the electrolyte content of sweat is in part under control of the adrenal cortical hormones. Darling and co-workers[2] made the other significant contribution with their finding of elevated concentrations of sodium and chloride in the sweat of children with cystic fibrosis of the pancreas. Conn and others[3,4,5] have suggested employing sweat electrolyte concentration as an index of adrenal cortical function. The "sweat test" has become routine in the diagnosis of cystic fibrosis and there are indications that a study of the energetics of the sweat gland may be helpful in understanding the derangement elsewhere in the body in this disease.[6]

The emphasis to date has been mainly on the excretion of sodium, chloride, and potassium by the sweat gland. Urea and lactic acid have also been studied extensively, and though other substances

found in sweat are mentioned in scattered reports, a great deal of attention has not been focused on them. One group of substances which has received relatively little attention is that of the amino acids. Most of the reports in the last few years have been by Japanese workers.[7,8] In all the reports sweat amino acids have been determined by modifications of the Folin technique. Chinard and Van Slyke[9] found that values obtained for amino acid nitrogen in plasma by the photometric procedure differed considerably from results obtained by the more accurate ninhydrin manometric method. Only 20% of the values were within ± 5%, 54% deviated by more than ± 15%, and some deviations exceeded 400%. This work was undertaken with two objectives: to determine sweat amino acid levels by the Van Slyke manometric ninhydrin method, and to determine whether the abnormalities of the sweat mechanism in cystic fibrosis affect the excretion of amino acids by this route.

METHODS

Fifteen children, ranging in age from $2\frac{1}{2}$ to 13 years, were used in this study. Seven were normal children; the other eight were known to have cystic fibrosis (one was proven by the sweat test for this experiment). The subjects had had no food intake for at least two hours, and usually four to five hours prior to the start of the sweat test. Clear fluids were allowed ad lib.

The subject was placed in a chamber of 50% humidity and at a temperature of 31-34°C. After a period of 15-30 minutes, an area on the abdomen above the umbilicus was washed with alcohol and distilled water, then dried. A 2 by 3 inch gauze square, previously eluted with distilled water to remove all contaminants, dried, and weighed in a flask, was then applied to the prepared area. (At all times during the procedure either rubber gloves or forceps were used in handling the gauze.) The gauze was covered with a sheet of waterproof plastic, which was fixed to the skin with waterproof tape. After $1\frac{1}{2}$-2 hours, the gauze was removed, replaced in the closed vessel where it had been preweighed, and the amount of sweat obtained was determined by weighing. In two subjects, DH and JH, the rate of sweating was sufficiently brisk so that the gauze was removed at the end of 1 hour and a second gauze was inserted. These were treated as separate specimens and analyzed individually.

A measured volume of distilled water, sufficient to cover the gauze, was added to the vessel, which was then placed overnight in

a refrigerator. A crystal of thymol was added to prevent bacterial decomposition.

After elution the gauze was wrung out and the elution fluid analyzed for amino nitrogen by the submicro or micro method described by Van Slyke, MacFadyen, and Hamilton[10] for analysis of urine. Two cc of sweat of approximately 1:10 dilution were placed in a ninhydrin reaction vessel, acidified, and buffered at pH 2.5. CO_2 was expelled by boiling. The sample was cooled and 100 mg of ninhydrin added. The vessel was at once closed, evacuated, and then heated in a boiling water bath for 8 minutes. Following this the CO_2 was transferred to the Van Slyke-Neill chamber and the P_{CO_2} determined. Two cc of elution fluid from a clean gauze square were used in preparing a blank. All but two samples were run in duplicate.

Urease was used in preparing the first several samples, but omitted therafter. Comparison of a sample run with and without the addition of urease showed no significant difference, and it was felt that the concentration of urea in sweat was sufficiently close to that of plasma (1.7 times the concentration in plasma, as shown by Schwartz[11] and by Gochberg[12]) that it need not be removed. For those samples on which urease was used, urease was also added to the blank.

All but one of the sweat samples were analyzed for chloride by a modification of the polarographic method of Zimmerman and Layton.[13] In a few instances, sodium, potassium, and lactate determinations were also done. Sodium and potassium were analyzed by flame photometry,

and lactate by the colorimetric method of Barker and Summerson.[14]

Plasma amino acid levels were obtained on six of the subjects, two normals and four cystics. The blood sample was drawn midway through the period of sweating, placed in an oxalate bottle, and quickly centrifuged. The plasma was drawn off and analyzed by the submicro method of Hamilton and Van Slyke.[15] Duplicates were run on each sample.

Two dimensional paper chromatography was carried out on several of the specimens by the method described by Block[16] using phenol-water and butanol-acetic-acid-water. The spots were developed by ninhydrin.

RESULTS

The accompanying table shows the values obtained in milligrams of α-amino nitrogen in the sweat of the individuals studied. In the case of DL the duplicates were in such poor agreement that the results were not considered reliable enough for inclusion. The second hour specimen from JH, after dilution, did not contain sufficient amino acid nitrogen to be measured, and before this could be ascertained, the sample was exhausted.

The mean value of the 15 specimens analyzed was 17.2 mg % α-amino nitrogen. The mean for the normal controls was 17.3 mg %, with a range of 5.2-32.0 mg %. The mean for the cystics was 17.0 mg %, with a range of 7.2-39.0 mg %.

Analysis of the two blood samples from normal subjects gave values of 3.7 and 5.1 mg % α-amino nitrogen (mean 4.4). The four samples drawn from cystics showed levels of 3.6, 3.8, 4.9, and 5.6 mg % (mean 4.5). The mean for all six samples was 4.5 mg %. There was no correlation between the levels of amino nitrogen in the blood and in the sweat of these subjects.

The values for sweat chloride concentration, and, where done, for the concentrations of sodium, potassium, and lactate are also presented in Table I. No correlation existed between the concentration of amino acids and the concentration of any of these elements, nor could any correlation be demonstrated between the sweat rate and the amino acid concentration. However, in both

instances where first and second hour specimens were obtained separately, the second hour specimen had a lower amino nitrogen concentration than did the first hour specimen.

Paper chromatography revealed identical amino acid patterns for the sweat of cystics and normals. Sufficient standards to permit identification of the spots have not yet been run.

DISCUSSION

The literature on the contents of various elements in the sweat is replete with contradictions. Unfortunately, the work reported herein adds one more contradictory report to the subject. The values for total amino nitrogen were spread over a wide range, but were consistently higher than those previously reported, and higher than the levels found in blood. Talbert and Haugen,[17] from 28 experiments on 12 different subjects, reported a range of 1.66-4.76 mg % in sweat. Itoh and Nakayama[7] found a range of 1.1-10.2 mg %, and Araki and Ando[8] a range of 3.6-6.2 mg %. It is difficult to reconcile the higher values obtained in this study. Although Chinard and Van Slyke[9] demonstrated (on plasma) that the ninhydrin method is more accurate than the colorimetric method, the differences here are of too great a magnitude to be explained solely on the basis of technique. In addition, on one sample (DH) analyzed by both the ninhydrin and the photometric method,[18,19] values of 29.3 mg % and 28.3 mg % respectively were obtained. However, a few additional factors deserve mention. Both Itoh and Araki found that the concentration of amino nitrogen decreased as the rate of sweating increased. A similar trend was observed in this study. The room temperature here was considerably lower than that in either of the Japanese studies (40-45°C) and the rate of sweating slower. This then may be a contributory factor. In addition, Araki used permutit to remove NH_3 from the sweat prior to analysis, and this agent is

known to remove some amino acids as well.[20,21]

One piece of work which supports these results is that done by Hier et al.[22] Using a microbiological assay for ten individual amino acids they found the total for these amino acids in sweat to be twice that in plasma. Their analyses included the same amino acids which Araki and Ando found to be present in greatest concentration.

The results here for plasma amino nitrogen are very close to those of Cramer and Winnick[23] (2.3-7.3 mg %, mean 4.2 mg %) and comparable to those found by Araki and Ando for serum (5.0-8.8 mg %), since serum levels are increased by the release of amino acids during the clotting process.[23]

It is interesting to speculate on the mechanism of amino acid excretion by the sweat gland. It is apparent that an entirely different process from that utilized in the transport of either sodium and chloride or urea is involved here. The concentration of amino acids varies inversely with the rate of sweating. Sodium and chloride concentrations, on the other hand, vary directly with the sweat rate,[24,25] while urea concentration is unchanged.[11] Sodium and chloride in sweat are affected very little by the blood levels of these substances, and some of this change is probably mediated through the adrenal cortex. Sweat urea seems to be constantly proportional to the blood urea, in a ratio of 1.7 to 1. The amino acids of sweat seem unrelated to blood levels,[22] although Araki did find that there was an increase in the excretion when the blood

levels of a single amino acid (Na glutaminate) were raised. The best evidence for a different mechanism, though, is the fact that no difference could be detected between the normals and the cystics in the excretion of amino acids in sweat.

Another finding must be considered in trying to understand the excretion mechanism. Arginine, histidine, threonine, and tyrosine are present in higher concentrations in sweat than in plasma.[22] Hier found the levels of isoleucine, leucine, lysine, phenylalanine, tryptophane, and valine equal in blood and sweat. Cystine and methionine are less concentrated in sweat than in blood. A comparison of sweat and urine may not be strictly accurate, especially since Talbert and Haugen could find no correlation in the amounts excreted by these two routes, but it is of interest nevertheless that Pitts[26] found arginine reabsorbed by the kidney least of four amino acids he studied (glycine, alanine, glutamic acid, and arginine).

In view of the evidence presented to date, it is unlikely that amino acids are excreted by a simple diffusion process. One possible mechanism may be that there is active transport of the amino acids into the sweat solution. The limiting factor in excretion would be at this point, and not in the reabsorptive process. This explanation would account for a decreased concentration with increased flow rates. The differential gradients between blood and sweat levels of the various amino acids may be due to some (e.g. arginine, histidine) faring better in competition for the transport system than others

(e.g. cystine). The reason for the successful competition of particular amino acids remains obscure at this time, although it may be worth noting that arginine and histidine are basic in reaction, while cystine is acid. It is obvious that further study of this subject is required to obtain a complete explanation.

SUMMARY

1. Total amino acid nitrogen concentration of sweat was determined in six normal children and eight children with cystic fibrosis of the pancreas using the ninhydrin-CO_2 determination.

2. No differences were found in the sweat amino acids of normals and cystic fibrosis patients.

3. A discussion of factors influencing sweat amino acid composition is presented.

Table 1

SUBJECT	AMOUNT OF SWEAT (Gm.)	DURATION OF SWEATING (min.)	SWEAT					BLOOD
			Amino N (mg. %)	Cl (mEq./L.)	Na (mEq./L.)	K (mEq./L.)	Lactate (mg. %)	Amino N (mg. %)
normal controls								
DH #1	1.6195	60	29.4	6.7				
DH #2	0.4582	60	14.4					
LS	0.9873	120	5.2	14.5	10.4	8.81		
KG	0.5431	120	9.0	16.4	14.6	21.2	45.4	
DL	0.4749	120		16.5	22.0	8.2		3.7
PM	0.3500	120	21.4	16.7	15.6	11.9		
PD	0.6138	95	10.0					5.1
MB	0.9710	105	32.0	11.8				
		MEAN	17.3					4.4
cystic fibrosis								
JH #1	1.2023	65	7.2	132.3				3.8
JH #2	0.2133	35	Insuf.					
EW	0.2595	60	39.0	79.0	80.4	13.9	136	3.6
DD	0.0305	165	15.4	177				5.6
KP	0.7573	105	10.7	112.7				
JL	0.5887	90	16.4					
RH	0.9790	115	11.2	134.5				
MD	0.7964	105	13.3	112.5				4.9
EF	0.9122	115	23.0	114.6				
		MEAN	17.0					4.5

BIBLIOGRAPHY

1. Conn, J.W., Johnston, M.W., and Louis, L.H. Acclimatization to Humid Heat: A Function of Adrenal Cortical Activity. J. Clin. Invest., 25: 912, 1946.

2. Darling, R.C., DiSant'Agnese, P.A., Perera, G.A., and Anderson, D.H. Electrolyte Abnormalities of the Sweat in Fibrocystic Disease of the Pancreas. Am. J. Med. Sci., 225: 67, 1953.

3. Conn, J.W., Louis, L.H., Johnston, M.W., and Johnston, B. The Electrolyte Content of Thermal Sweat as an Index of Adrenal Cortical Function. J. Clin. Invest., 27: 529, 1948.

4. Conn, J.W. Electrolyte Composition of Sweat. Arch. of Int. Med., 83: 416, 1949.

5. Locke, W., Talbot, N.B., Jones, H.S., and Worcester, J. Studies on the Combined Use of Measurements of Sweat Electrolyte Composition and Rate of Sweating as an Index of Adrenal Cortical Activity. J. Clin. Invest., 30: 325, 1951.

6. Gochberg, S.H., and Cooke, R.E. Sweat Gland Physiology. To be published.

7. Itoh, S., and Nakayama, T. Amino Acids in Human Sweat. Jap. J. Physiol., 2: 248, 1952.

8. Araki, Y., and Ando. S. Urea, Amino Acid, and Ammonia in Sweat. Jap. J. Physiol., 3: 211, 1953.

9. Chinard, F.P., and Van Slyke, D.D. Comparison of a Modified Folin Photometric Procedure and the Ninhydrin Manometric Method for the Determination of Amino Acid Nitrogen in Plasma. J. Biol. Chem., 169: 571, 1947.

10. Van Slyke, D.D., MacFadyen, D.A., and Hamilton, P.B. The Gasometric Determination of Amino Acids in Urine by the Ninhydrin-Carbon Dioxide Method. J. Biol. Chem., 150: 251, 1943.

11. Schwartz, I.L., Thaysen, J.H., and Dole, V.P. Urea Excretion in Human Sweat as a Tracer for Movement of Water within the Secreting Gland. J. Exp. Med., 97: 429, 1953.

12. Gochberg, S.H. Whole Body Sweat Studies in Cystic Fibrosis of the Pancreas. Thesis for M.D. Degree at Yale University School of Medicine, New Haven, 1956.

13. Zimmerman, W.J., and Layton, W.M. A Polarographic Method for Determination of Blood Chloride. J. Biol. Chem., 181: 141, 1949.

14. Barker, S.B., and Summerson, W.H. The Colorimetric Determination of Lactic Acid in Biological Material. J. Biol. Chem., 138: 535, 1941.

15. Hamilton, P.B., and Van Slyke, D.D. The Gasometric Determination of Free Amino Acids in Blood Filtrates by the Ninhydrin-Carbon Dioxide Method. J. Biol. Chem., 150: 231, 1943.

16. Block, R.J., Durrum, E.L., and Zweig, G. A Manual of Paper Chromatography and Paper Electrophoresis. Academic Press, Inc. New York, 1955.

17. Talbert, G.A., and Haugen, C. Constancy of Amino Acids in the Sweat of Normal Individuals. Am. J. Physiol., 81: 510, 1927.

18. Frame, E.G., Russell, J.A., and Wilhelmi, A.E. The Colorimetric Estimation of Amino Nitrogen in Blood. J. Biol. Chem., 149: 255, 1943.

19. Russell, J.A. Note on the Colorimetric Determination of Amino Nitrogen. J. Biol. Chem., 156: 467, 1944.

20. Hawk, P.B., Oser, B.L., and Summerson, W.H. Practical Physiological Chemistry. The Blakiston Co., Inc. New York, Thirteenth Ed., p. 899.

21. Block, R.J. et al., op. cit., p. 84.

22. Hier, S.S., Cornbleet, T., and Bergeim, O. The Amino Acids of Human Sweat. J. Biol. Chem., 166: 327, 1946.

23. Cramer, F.B., Jr., and Winnick, T. Amino Acid Nitrogen of Normal Human Plasma. J. Biol. Chem., 150: 259, 1943.

24. Schwartz, I.L., and Thaysen, J.H. Excretion of Sodium and Potassium in Human Sweat. J. Clin. Invest., 35: 114, 1956.

25. Darling, R.C. Some Factors Regulating the Composition and Form of Human Sweat. Arch. Phys. Med., 29: 150, 1948.

26. Pitts, R.F. A Comparison of the Renal Reabsorptive Processes for Several Amino Acids. Am. J. Physiol., 140: 535, 1944.

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